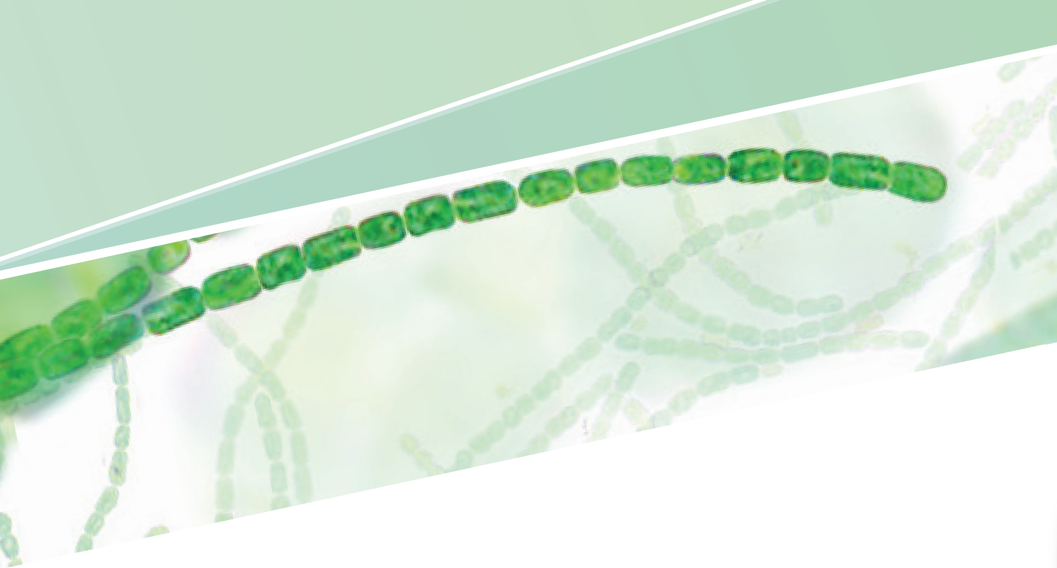

Abstracts and program

5 | National Cyanobacteria WORKSHOP

Emerging cyanobacterial research and its practical application

NCW5
2016
BRISBANE
29–30 September

Emerging cyanobacterial research and its practical application

Abstracts from the Fifth National Cyanobacterial Workshop

Brisbane, 29–30 September 2016

This National Cyanobacterial Workshop was hosted by the Australian Rivers Institute, Griffith University, Queensland.

Acknowledgement of Country

We acknowledge the people who are the traditional custodians of this land, pay respect to Elders, past and present, and extend that respect to other Aboriginal and Torres Strait Islander peoples. The South Bank campus is situated on the land of the Yuggera and Turrbal peoples.

Organisation of the workshop was led by:

Professor Michele Burford – Executive Deputy Director, Australian Rivers Institute, Griffith University

Other members of the committee:

Dr Philip Orr – Griffith University and Chair of the NCW5 Organising Committee

Dr Anusuya Willis – Griffith University

Mrs Karen Reardon – Queensland Health Forensic and Scientific Services

Dr Barbara Sendall – Queensland Health Forensic and Scientific Services

Dr Glenn McGregor – Queensland Department of Science, Information Technology and Innovation

Dr Wasantha Wickramasinghe – ENTOX/ University of Queensland

Ms Carla Littlejohn – Limnologic

Ms Angela Gackle – Water Research Australia

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Australian Rivers Institute, Griffith University

SA Water

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Queensland Health Forensic and Scientific Services

Melbourne Water

National Research Centre for Environmental Toxicology, The University of Queensland

Seqwater

Water Research Australia Limited

Workshop Program – Day 1

7:30	Registration: Webb Centre (Building S02, Level 7, Rooms 7.16, 7.07)	
8.45	Move to Program Venue: QCA Lecture Theatre (Building S05, Level 2, Room 2.04)	
9:00	**Official welcome by Michele Burford**	
9:10	**Opening address – Ian Falconer**	
Session 1	**Session theme – Understanding for management. Chair Glenn McGregor**	
9:25	Anusuya Willis	*Cylindrospermopsis raciborskii* strain variation – implications for understanding blooms
9:40	Md Ashraful Islam	Effects of temperature on growth and responses to UVB of the toxic cyanobacteria *Anabaena circinalis* and *Microcystis aeruginosa*
9:55	Barbara Sendall	What lies beneath? Prevalence of toxigenic *Scytonema* in benthic freshwater habitats in south-east Queensland
10:10	Joao Pereyra	Molecular and physiological survey of saxitoxin-producing *Anabaena circinalis* isolated from geographically distinct regions of Australia
10:30	**Morning Coffee: Webb Centre (Building S02, Level 7, Rooms 7.16, 7.07)**	
Session 2	**Session theme – Monitoring. Chair Larelle Fabbro**	
11:00	**Keynote Speaker David Hamilton**	**Connecting science, modelling capability and management of harmful cyanobacteria blooms**
11:30	Lee Bowling	A bloom of *Chrysosporum ovalisporum* in the Murray River, 2016
11:45	Tapas Biswas	River Murray phytoplankton monitoring program
12:00	Nijoy John	Toxigenicity assessment of the Murray River bloom using a rapid and automated multiplex tandem- polymerase chain reaction
12:15	Tim Malthus	A low cost near surface sensor for algal bloom detection
12:30	John Runcie	An autonomous cyanobacteria monitoring system
12:45	**Lunch: Webb Centre (Building S02, Level 7, Rooms 7.16-7.17)**	
Session 3	**Session theme – Treatment and control. Chair Nick Crosbie**	
13:45	Arash Zamyadi	Fate of harmful cyanobacteria and their associated metabolites in Australian water treatment plants
14:00	Amelie Prevost	Coagulation impacts on biological removal of two cyanobacteria metabolites: 2-methyl-isoborneol and geosmin
14:15	Simon Tannock	Enhanced diatom growth as a method for reduction of cyanobacteria in wastewater treatment lagoons in SE Queensland
14:30	Bradford Sherman	The impact of draft-tube mixers on thermal stratification dynamics in Googong reservoir
14:45	Man Xiao	Differences in cyanobacterial strain responses to light and temperature drives species plasticity
15:00	Michele Burford	Global HAB update
15:15	**Afternoon Coffee: Webb Centre (Building S02, Level 7, Rooms 7.16, 7.07)**	
Session 4	**Session theme – Monitoring. Chair Susie Wood**	
15:45	Jonathan Puddick	Fine-scale cryogenic sampling of planktonic microbial communities: application to toxic cyanobacterial blooms
16:00	Andrea Paparini	Next generation monitoring of water quality
16:15	Mark Van Asten	The development of the Phytoxigene™ CyanoDTec test from research to implementation as a cyanobacteria screening tool for prediction, monitoring and management of harmful algal blooms in public water supplies
16:30	**Poster presentation session**	

16:50	**Wrap up of Day 1**
17.15	Poster session and pre-dinner socialising *66 on Ernest* (*66 Ernest St,* South Brisbane)
19:00	Dinner at *66 on Ernest*

Workshop Program – Day 2

8:00	Coffee/tea: Webb Centre (Building S02, Level 7, Rooms 7.16, 7.07)	
8.45	Move to Program Venue: QCA Lecture Theatre (Building S05, Level 2, Room 2.04)	
Session 5	**Session theme – Management. Chair Maree Smith**	
9:00	**Keynote Speaker Rachael Poon**	**Applying Cyanobacteria Risk Management – A Victorian Perspective**
9:30	Cameron Veal	Managing cyanobacterial toxin risks to recreators: A case study for *Cylindrospermopsis raciborskii* dominated inland lakes in south east Queensland
9:45	Michele Burford	Should we be controlling nitrogen or phosphorus to manage cyanobacterial blooms? Lessons from studies of *Cylindrospermopsis raciborskii*
10:00	Klaus Joehnk	Sensing, simulating and predicting harmful algal blooms in inland waters
10:15	Ian Falconer	Cyanobacterial problems in an iconic lake – Lake Burley Griffin, Canberra
10:30	**Morning Coffee: Webb Centre (Building S02, Level 7, Rooms 7.16, 7.07)**	
Session 6	**Special Management Session. Chair Michele Burford**	
11:05		
12:05	**Lunch: Webb Centre (Building S02, Level 7, Rooms 7.16, 7.07)**	
Session 7	**Session theme – Understanding. Chair Paul Wright**	
13:00	Tara McAllister	The interactive effects of flow and nitrate supply on *Phormidium* accrual
13:15	Laura Kelly	Enumerating toxic *Phormidium autumnale* in environmental samples
13:30	Ian Stewart	Acute oral toxicity of deoxy-cylindrospermopsin in male Balb/c mice
13:45	Ann Chuang	Intraspecific variation in growth, morphology and toxin quotas for the cyanobacterium, *Cylindrospermopsis raciborskii*
14:00	Susie Wood	Risky rivers: Identifying river susceptibility and factors that promote benthic *Phormidium* proliferations
14:15	Glenn McGregor	A novel nodularin producing cyanobacterium from Edgbaston Reserve, north-eastern Australia
14:30	**Wrap up of Day 2**	
15:00	**Afternoon tea and departure: Webb Centre (Building S02, Level 7, Rooms 7.16, 7.07)**	

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Welcome Address – Michele Burford

The organising committee for the fifth National Cyanobacteria Workshop would like to welcome you to Brisbane on 29–30 November 2016. It is great to have so many water managers, researchers and others interested in cyanobacterial bloom problems present at our workshop. The organising committee has worked hard to deliver what is hopefully an interesting program combining presentations, a poster session, a discussion forum and social events. For visitors from other parts of Australia and overseas, we hope that you will find time to visit the sights of Brisbane and beyond.

Australia continues to face many challenges with managing and predicting cyanobacterial blooms, as highlighted by the recent major bloom along a large stretch of the Murray River last summer. The opportunity for interaction and communication between those dealing directly with problems associated with blooms, and those researching cyanobacteria is critical to ensuring that we can improve our ability to manage blooms now and in the future.

The organising committee hopes your time at the workshop is enjoyable and productive, and we look forward to meeting new and previous participants.

Professor Michele Burford
Executive Deputy Director
Australian Rivers Institute
Griffith University

ORAL ABSTRACTS – Day 1

Cylindrospermopsis raciborskii strain variation – implications for understanding blooms

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Abstract: Blooms of *Cylindrospermopsis raciborskii* can have highly varying toxin yields, with differences in the average toxin cell quotas occurring temporally and spatially. Our recent work has investigated toxin cell quotas and variations in morphology and physiology of multiple strains and how this can affect bloom population dynamics.

Toxin cell quotas of strains within a population can vary significantly, from 0 - ≥250 fg per cell. These toxin quotas are constant for each strain: cylindrospermopsin is constitutively produced irrespective of changing conditions. The result is that the toxin yield of a population composed of multiple strains each with a different toxin cell quota can vary considerably, depending on the proportion of the strains. This explains the disconnect between cell numbers and toxin yield, and also why the average cell quota of a population can differ substantially.

C. raciborskii strains also vary in morphology and physiology, with different cell size, growth rates, and nutrients use strategy. It is likely that these variations between strains regulate their different responses to environmental stimuli; with each strain having a distinct environment niche, or growth optimum. This means that changes in the environment can lead to changes in the proportion of strains, and hence lead to different toxin yields.

Current methods for monitoring bloom populations include cell counts, toxin gene counts and toxin yield measurement. Despite differences in toxin cell quotas, the cylindrospermopsin gene biosynthesis cluster is identical in all Australian strains tested, and is unable to account for differences in toxin cell quota, reflecting the disconnect between gene copy numbers, cell numbers and toxin yield.

The implications of these results for bloom management is that without methods for identifying strain toxin quotas it is not possible to predict toxin yield from cell enumeration. Improved monitoring and prediction of blooms requires greater understanding of the physiological variation between strains in their response to environmental stimuli, so that population dynamics can be more accurately modelled.

Key words: *Cylindrospermopsis raciborskii*, cylindrospermopsin, ecophysiology, management, strains, strain variation, toxin yield, toxin quota

Effects of temperature on growth and responses to UVB of the toxic cyanobacteria *Anabaena circinalis* and *Microcystis aeruginosa*

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Abstract: Temperature and solar radiation are two of the most important environmental factors that directly or indirectly influence algal growth. Global temperatures are rising as a consequence of elevated atmospheric CO_2 levels and, due to the depletion of the stratospheric ozone layer, the transmission of UVB radiation (280–320 nm), to the Earth's surface has been increasing for many decades. Elevated UVB levels are expected to persist until at least the middle of the century. The effects of temperature and UVB on the photosynthetic response of algae and cyanobacteria is an important issue due to the role of these organisms at the base of food chains and their potential to cause economically damaging blooms. To understand the interactive effects of temperature and UVB radiation, we grew cultures of *Anabaena circinalis* and *Microcystis aeruginosa* at either 25 °C or 30 °C and then exposed to an acute dose of UVB (1.4 W m^{-2}) until the effective quantum yield of Photosystem II (Fv'/Fm') declined to an asymptote. Rates of damage (*k*) and repair (*r*) were calculated from the kinetics of change in Fv'/Fm'. Recovery rates (*R*) were investigated subsequently by exposing cells to weak PAR fluxes (10 µmol photons m^{-2} s^{-1}) and following the rise in Fv'/Fm'. For both UV exposure and recovery experiments, Fv'/Fm' of PSII was measured every 5 minutes using a PHYTO-PAM fluorometer. To determine the presence of UV absorbing compounds (UVACs) cells were resuspended in 1 ml cold (5 °C) tetrahydrofuran:methanol (20:80, v/v) and the absorption spectra of the extracts were measured between 200 and 700 nm. Differences in growth curves were found in strains growing under the two temperature regimes. *A. circinalis* (CS541) showed a similar growth pattern at both temperatures, with higher growth rates at 30 °C than at 25 °C ($p = 0.0233$). However, final cell concentrations and growth rates of *M. aeruginosa* (CS558) were higher at 25 °C than at 30 °C ($p = 0.0001$). Analysis of the estimates of *r* and *k* shows that *M. aeruginosa* exhibited relatively high *r* and *k* values, compared to *A. circinalis,* at both growth temperatures. However, both species didn't show didn't show any statistical difference in terms of *r* and *k* at two temperature treatments. Recovery rates (*R*) after UVBR exposure showed no statistical differences when compared both within and between species ($p > 0.05$) at the two temperature regimes. However, the *R* values were relatively high at 25 °C, in the case of both species, compared to 30 °C. By measuring absorption spectra we found *M. aeruginosa* mainly absorbs in the UVA region whereas *A. circinalis* absorbs in both the UVA and the UVB regions. This could be the reason why the damage rate of *M. aeruginosa* was higher than that of *A. circinalis.* It could be concluded that temperature may influence growth and bloom formation of cyanobacterial strains and different strains may respond differently to UVB and temperature interactions.

Key words: *Microcystis aeruginosa*, *Anabaena circinalis*, UVB radiation, repair rate, damage rate, recovery rate, photoinhibition

What lies beneath? Prevalence of toxigenic *Scytonema* in benthic freshwater habitats in south-east Queensland

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Abstract: Surveys of benthic cyanobacteria from diverse freshwater aquatic habitats in south-east Queensland revealed a filamentous cyanobacterium putatively identified as *Scytonema crispum* Agardh ex Bornet et Flahault from a number of sites. Several strains were isolated and screened for the presence of five cyanotoxin genes. Some of these strains were found to have the *sxtA* gene, a known determinant for the production of saxitoxin (STX) and other paralytic shellfish toxins. Analysis of the three strains by HPLC confirmed they produced STX at concentrations between 2.8–18.9 mg g^{-1} dry weight. Phylogenetic analysis demonstrated that the sequence of the *sxtA* gene in the STX-producing strains was very similar to STX-producing *Scytonema* cf. *crispum* strains isolated from similar habitats in New Zealand (NZ). Similarly, phylogenetic analysis of 16S rRNA sequences grouped the three Australian strains with strains from NZ, together forming a well-supported monophyletic clade that was distinct from other *Scytonema* species and closely related genera. Characterisation of another conserved gene, *nifH* (which encodes an enzyme required for nitrogen fixation), confirmed the monophyletic origin of the Australian and NZ strains compared with other *Scytonema* sequences. Interestingly, from the same locations in which STX-producing strains were found, we also isolated strains of *S. crispum* that did not have the *sxtA* gene, nor any other cyanotoxin genes. Toxigenic and non-toxigenic strains were morphologically indistinguishable from one another. *Scytonema* is generally a metaphytic and/or benthic species that forms mats which may detach and float upwards in the water column or become deposited along the edges of rivers and lakes, posing a risk to recreational users and animals via ingestion and/or direct skin contact. As it is currently unknown whether benthic STX-producing *Scytonema* release STX into the water, the risk they may pose in drinking water bodies such as large reservoirs is difficult to assess. However, in the absence of surveillance for this newly identified STX-producer there is an unrecognised risk of cyanotoxin exposure to humans and animals.

Key words: phylogeny, taxonomy, cyanotoxin, saxitoxin, paralytic shellfish poison, benthic, *sxtA*, 16S rRNA, *nifH*

Molecular and physiological survey of saxitoxin-producing *Anabaena circinalis* isolated from geographically distinct regions of Australia

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Abstract: Monitoring of waterways for potential contamination of drinking water is an ongoing cost. Previous studies have reported different isolates of toxic *Anabaena circinalis* producing different types of saxitoxin at different amounts, but it is not understood how. To assess the characteristics that may be responsible for the variance, a molecular and physiological survey of 29 Australian *A. circinalis* isolates was conducted. We assessed differences in cell length, cell width, average number of cells per filament, the presence or absence of saxitoxin (*sxt*) genes and amount of toxin produced.

The 29 isolates were collected from 12 locations around Australia for morphological characterisation and revealed cell size ranged from 5 µm to 11 µm, while filament length ranged from 5 µm to 605 µm. There was no correlation between the size of the isolates and their reported toxicity or geographical location. Genomic DNA was extracted and used for the molecular screening for the presence of several *sxt* genes involved in core saxitoxin biosynthesis (*sxtA*) and structural tailoring enzymes (*sxtL*, *sxtN*, *sxtSUL* and *sxtX*). Overall, we identified 9 out of 29 strains that harboured the *sxt* gene cluster and all tailoring genes except *sxtX*. Furthermore, production of saxitoxins was correlated with the presence of the *sxt* cluster by quantitative pre-column oxidation high performance liquid chromatography with fluorescence detection (HPLC-FLD). Isolates produced different saxitoxin analogues at different levels.

Correlation of physiological characteristics with toxin profile/quantity from distinct sites around Australia will aid the management of these at-risk areas by potentially giving insight into the molecular control or physiological characteristics of toxin production.

Key words: gene, saxitoxin, paralytic shellfish toxins, *Anabaena circinalis*

Keynote Address 1 – David Hamilton

Connecting science, modelling capability and management of harmful cyanobacteria blooms

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Abstract: Understanding, predicting and managing harmful cyanobacterial blooms in lake ecosystems challenges our capacity to connect disciplines and scales. At the disciplinary level, significant progress has been made in understanding the roles of buoyancy, small-scale hydrodynamics and taxon-specific features that are important in cyanobacterial bloom formation. The emerging challenge is to link together the relevant physiological and hydrodynamic drivers that lead to blooms. Models have been able to provide highly specific information on several characteristics of cyanobacteria such as nitrogen fixation and buoyancy regulation, but such features are mostly ignored or factored in empirically when these models are applied at the whole lake scale. At the management level there are also well developed protocols and guidelines to mitigate risks to human health from cyanobacterial blooms but the often tenuous links between health authorities, managers and scientists can lead to a disconnect between the theory and practice of bloom management. Scale compounds these challenges. For example, improved understanding of cyanobacterial toxin production has required measurement at progressively finer temporal and spatial scales. With new and accessible tools such as cryo-preservation of water samples, transcriptome analysis of cyanobacteria, phycocyanin sensors and remote sensing, I argue that we have the tools available to provide a far more complete description of existing cyanobacterial population dynamics and toxin production at multiple scales. Models can assist with this scaling but at the ecosystem level they require substantial upgrade of process descriptions to match what is already known about the physiological ecology of cyanobacteria. The breakthrough in improving predictions of when and where harmful cyanobacterial blooms will occur – ultimately what is of most interest and value to water managers – is most likely to come through improving connections at the interface of disciplines and being able to successfully harness the multiple emerging technologies that are increasingly accessible.

Key words: cyanobacterial toxins, phycocyanin, remote sensing, models, scaling, lakes

A bloom of *Chrysosporum ovalisporum* in the Murray River, 2016

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Abstract: An unusual bloom of *Chrysosporum ovalisporum* (basionym *Aphanizomenon ovalisporum*) occurred in the Murray River, NSW, from mid-February to early June 2016. At its greatest extent in April and May it extended along the river from Lake Hume to Lock 8 and also throughout the Edward, Wakool and Niemur River distributary system in NSW, a combined river length of ca. 2360 km. It also extended into distributary systems in Victoria, for example Gunbower Creek. Bloom densities at times exceeded 40 $mm^3\ l^{-1}$, and C. *ovalisporum* usually comprised >99% of the total bloom biovolume at most locations sampled. The origins of the bloom were most likely Lakes Hume and Mulwala on the upper Murray River, with cyanobacterial infested water released from them contaminating the river systems downstream.

Previously, C. *ovalisporum* has occurred infrequently in NSW, and then mostly at low abundance and as a minor component of the total cyanobacterial communities. Blooms from Tweed Heads (NSW) and Hervey Bay (Qld), as well as from several Mediterranean countries have however been documented in the literature. These report most blooms occurring at water temperatures >25°C and all being toxic, producing cylindrospermopsin.

Why the 2016 Murray River bloom was of C. *ovalisporum* and not of the more commonly occurring Australian bloom forming genera (*Dolichospermum*, *Microcystis*, *Cylindrospermopsis*) can only be speculated, but may be due in part to above average temperature in the southern Murray Darling Basin in late summer and autumn 2016. However, the bloom differed from ecophysiological conditions reported as optimal for the species, including occurring at much lower water temperatures, especially from April to June. In addition, testing by both chemical and genetic methods found toxins below detection and very low copy numbers of genes responsible for toxin biosynthesis, including *cryA*.

Key words: cyanobacterial bloom, biovolume, climate change, toxicity

River Murray phytoplankton monitoring program

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Abstract: The River Murray and its tributaries carry hundreds of algal and cyanobacterial species (phytoplankton). The routine assessment and collection of data relating to phytoplankton in the River Murray and its major tributaries was initiated by the River Murray Commission in 1980. It was continued by the former Murray-Darling Basin Commission and now managed by the Murray-Darling Basin Authority (MDBA). The River Murray phytoplankton monitoring program (RMPMP) has a crucial role in fulfilling the statutory requirements under Clauses 44-46 of Schedule 1 of the Water Act 2007 and requirements of the Basin Plan 2012.

Algae and cyanobacteria play a major role in the carbon fixation and food web dynamics of the River Murray, and are a key driver of its in stream ecosystem. The primary objective of the RMPMP is to provide long-term data on the algal populations of the River Murray, so that changes in the abundance and community composition may be identified and related to changes in the river environment. The second aim of the program is to increase the scientific understanding of phytoplankton in the River.

The database generated through this program is considered by many to be an environmental resource of world standard, as it records the phytoplankton history of the river. Linking this data to water quality and hydrological databases provides an integrated picture of river health over time. No other programs in the Basin monitor the broader phytoplankton community, as the sampling undertaken by States overwhelmingly monitors cyanobacteria only.

Key findings to date include:

- Frequency of cyanobacterial blooms within the River Murray has not changed markedly since 1980, remaining within the 1–4 year range,
- Frequency of blooms is generally higher upstream than downstream,
- Duration of individual algal blooms increased at the upstream sites post 2000 compared to what was recorded in the 1980s,
- Consistent and statistically significant increases in counts have been observed across almost all sites during 1994–2008 (during millennium drought),
- Potentially toxic *Dolichospermum circinale* counts have increased strongly across all sites over the period 1994–2008, and
- A species previously infrequently found in the river, *Chrysosporium ovalisporum*, bloomed in 2016 for the first time in the Murray.

Key words: phytoplankton, River Murray, monitoring, cyanobacteria bloom, blue-green algae, *Chrysosporium ovalisporum*

Toxigenicity assessment of the Murray River bloom using a rapid and automated multiplex tandem-polymerase chain reaction

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Abstract: Cyanobacterial blooms are a major water-quality issue affecting management of surface waters globally. In addition to impacting water palatability, these blooms have the potential to produce a variety of hepato- and/or neurotoxic compounds (cyanotoxins) that represent a potential risk to wildlife, livestock, domestic animals and the general public. A major challenge in the management of these blooms is that their genetic potential to produce cyanotoxins (toxigenicity) is based on the presence or absence of the genes responsible for cyanotoxin production and cannot be determined by microscopy.

Here, we describe the use of a semi-automated, quantitative PCR (Multiplex-tandem PCR) to detect the toxigenicity of a recent (2016) bloom event affecting a large section of the Murray Darling Basin along New South Wales and Victoria. In total, 194 samples collected between 21-3-2016 to 9-5-2016 from 18 locations along the Murray River (from Wahgunyah, Victoria to Wemen, Victoria) were assessed by microscopic examination and then tested for the presence and copy number of genes essential for the production of each of the four major cyanotoxins of relevance to Australia (*i.e.*, cylindrospermopsins, microcystins, nodularins and saxitoxins). Microscopy data suggest that *Chrysosporum cf. ovalisporum* (a potential cylindrospermopsin producer) was the dominant cyanobacterium present in all samples, along with a variety of, typically non-toxic cyanobacterial species. In some locations, *Cylindrospermopsis raciborskii*, a known cylindrospermopsin producer, *Dolichospermum* sp. – a potential saxitoxin producer and *Microcystis* sp. – potential microcystin producers were also reported in small quantities. Based on MT-PCR testing, we detected microcystin (180/194), cylindrospermopsin (141/194) and saxitoxin (146/194) producing genes in 93, 73 and 75 % of the samples tested. By prevalence, MT-PCR indicates an evenly distributed bloom - with microcystins at 39 %, cylindrospermopsin at 30 % and saxitoxin at 31 %. By quantitative abundance, *Microcystis*-like *mcyE* gene was the most predominant (74 %) followed by cylindrospermopsin (19 %) and saxitoxin (7 %) producing genes. Moreover, the MT-PCR data indicates the presence of at least two major blooms. Cylindrospermopsin and microcystin producers were equally abundant and prevalent at sites downstream from Wahgunyah to Torrumbarry and microcystin producers were abundant from Pental to Wemen. Direct toxin detection by Enzyme linked Immunosorbent Assay (ELISA/ Abraxis kits) and High Performance Liquid Chromatrography (HPLC) confirmed the presence of these cyanotoxins, but at unexpectedly low levels considering the PCR results. Our results suggest that the Murray bloom was likely a composite of multiple either successional or independent blooms, distributed in at least two major zones along the river. We note higher than expected toxin gene counts in these blooms relative to the concentration of toxins detected in corresponding water samples and bacterial cell pellets and postulate a possible environment cause.

Key words: cyanobacteria, water quality, toxic blooms, cyanotoxin, cylindrospermopsin, microcystin, saxitoxin, ELISA, HPLC

A low cost near surface sensor for algal bloom detection

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Abstract: In Australia, algal blooms result in ~AU$250 million costs to affected communities per annum. Increases in potentially toxic cyanobacterial blooms are a particular concern, increasing threats to human and animal health. Whilst reliable, traditional field monitoring for algal blooms involving identification and cell counting takes time, can be limited in spatial extent, and risks chronic under-sampling of episodic processes like algal blooms. Additional tools are required to assist in more rapid and widespread bloom monitoring at temporal resolutions that allow for early detection of blooms to assist early warning for management intervention.

Field based sensor systems provide a useful complement to traditional water quality sampling methodologies. For bloom detection the most commonly deployed devices are based around the detection of algal pigments using fluorometric sensors. However, fluorometric systems may differ in measurement units and wavelengths used and readings may also be inaccurate, affected by previous phytoplankton light exposure, varying phycocyanin content as well as water turbidity and temperature. Accurate calibration is also critical. Such systems also have ongoing expenses related to instrument service, calibration and maintenance.

The lower the costs of a field sensor the more that can be deployed and the more widely dispersed measurements can be. CSIRO strategic research has explored the use of an inexpensive (~$1000) non-contact optical solution to bloom detection based on near surface reflectance measurement exploiting the same methods used in remote sensing. Installed at specific lake and river sites, the sensors could establish a continuously sensing network to provide an early warning of developing bloom and other water quality problems. Being non-contact prolongs the need for routine maintenance in comparison to in situ systems.

The presentation will report on the development and testing of the sensor, and the quality of data produced in relation to more expensive commercial in situ optical systems. The potential uses of such devices as algal bloom early warning systems will also be highlighted using Southeast Queensland examples.

Key words: sensors, spectral sensing, cyanobacteria, algal blooms, water quality, remote sensing, visualisation, information services, early warning, citizen science

An autonomous cyanobacteria monitoring system

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Abstract: Determining cell concentrations of toxic cyanobacteria in freshwater reservoirs, rivers and streams at concentrations lower than the ANZECC guideline threshold limit of 2000 cells/mL is needed for rapid detection of developing toxic algal blooms. An early warning of increasing cell concentrations will better enable prompt initiation of management actions designed to prevent rising in cell concentrations. If the detection of increasing cells counts is too late, remediation actions can be far less effective, hence the need for an autonomous device capable of detecting cell concentrations less than 2000 cells/mL. Here we report preliminary results from a prototype cyanobacteria monitor that examines specific spectral qualities of collected cells. Later versions of the device will be deployed in water bodies in Victoria where high cyanobacteria concentrations have previously been reported.

Key words: cyanobacteria, fluorescence, phycocyanin, water quality, algal bloom, autonomous monitoring, potable water

Fate of harmful cyanobacteria and their associated metabolites in Australian water treatment plants

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Abstract: In 2016, cyanobacterial blooms stretched over 1300 km of the Murray River and other water bodies in Queensland (QLD), New South Wales (NSW), Victoria (VIC) and South Australia (SA), demonstrating the ongoing challenges of cyanobacterial related issues for Australian water utilities. Understating the fate of harmful cyanobacteria, i.e. toxic cells and/or producers of taste and odour (T&O) compounds, and their associated metabolites during water treatment is necessary for their successful removal, protection of public health and customer satisfaction. The objective of this project is to systematically study the fate of cyanobacterial cells and their metabolites (including toxins, T&O and organic matter) in Australian full-scale water treatment plants.

Intensive sampling was conducted in two treatment plants in QLD, one plant in NSW, one treatment plant in VIC, and three plants in SA from January 2015 to April 2016. Samples were taken from the following locations within the studied plants, where available: 1) water intake, 2) after sludge supernatant reuse, 3) after pre-oxidation, 4) post addition of powdered activated carbon (PAC), 5) post clarification (sedimentation or dissolved air flotation), 6) post filtration, 7) post advanced oxidation processes (AOP), 8) post granular activated carbon (GAC), 9) post oxidation, and 10) from the sludge supernatant in sludge treatment basins or lagoons. Species identification, cell count, cell viability tests using flow cytometry, 3D fluorescence excitation emission matrix (FEEM) spectroscopy, liquid chromatography - organic carbon detection (LCOCD), toxin (microcystins, cylindrospermopsin, saxitoxins and anatoxins) and T&O compound (MIB and geosmin) analyses were conducted on all samples.

Results of this unprecedented monitoring campaign demonstrated the variation in dominant species across the studied regions. *Cylindrospermopsis* sp., *Microcystis* sp., *Pseudanabaena* sp. and *Dolichospermum crassum* were dominant species in QLD, NSW, VIC and SA, respectively. The maximum recorded cell numbers in the raw water was 3.15 x 10^6 cells/mL. Up to 27000 cells/mL were detected after clarification processes and a maximum of 1200 cells/mL were counted in treated water. Notably, 80% and 20% of cells (even with cells numbers lower than 5000 cells/mL) were viable after pre-oxidation using $KMnO_4$ and ozone respectively. Accumulation of viable cells within the clarifier sludge (64–92% viable cells), cell survival and growth during the water recovery from the sludge and backwash spent water, and toxin and T&O compounds (29 to 1200 ng/L) release by these cells and their impact on quality of recycled and treated water were documented for the first time. Comprehensive characterisation of cyanobacterial organic matter using FEEM and LCOCD analysis helped to identify key compounds affecting water treatability and process efficiency.

These results help to define the scale of cyanobacterial and their harmful metabolites monitoring and treatment challenges in Australian full-scale water treatment plants. Furthermore, the outcome of this study helps to outline the knowledge gaps in removal of cyanobacterial cells and their metabolites from water. These results were obtained under the ongoing ARC Linkage Project research strategy conducted at UNSW Australia with support of seven Australian water utilities, regulatory agencies and research organisations.

Key words: cyanobacteria, cyanotoxins, taste and odour, organic matter, water treatment, water recovery, sludge treatment, monitoring strategy

Coagulation impacts on biological removal of two cyanobacteria metabolites: 2-methyl isoborneol and geosmin

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Abstract: Taste and odour compounds in drinking water, aside from being aesthetically unpleasant can also be perceived by customers as an indication of the water being unfit for human consumption. 2-methyl isoborneol (MIB) and geosmin are two metabolites produced by a large array of cyanobacteria which impart musty or earthy flavours to the water. They are recalcitrant to removal by conventional water treatment practices forcing operators to use more costly processes such as activated carbon or oxidation. With detection thresholds as low as 4 ng/L for MIB and 9 ng/L for geosmin, the removal of these compounds is challenging. Biological degradation within existing rapid sand filters is potentially a cost efficient alternative for elimination of taste and odour compounds. However, in conventional water treatment biological filtration can be significantly impacted by upstream treatment processes, such as coagulation and oxidation. Understanding how such processes impact biological degradation is crucial to its widespread adoption. Here we investigate the influence of coagulation on biodegradation in three raw water samples from South Australian drinking water treatment plants. The coagulants aluminium sulphate and ferric chloride were tested at three different doses, and the resulting settled waters placed in bioreactors and spiked with MIB and geosmin. Settled waters from the water treatment plant were set up similarly for comparison. Biodegradation was monitored over 27 days. Post jar test settled waters were sampled for metal, hardness and nutrient analyses. MIB was found to be more recalcitrant to biodegradation than geosmin. Waters coagulated with ferric chloride achieved better metabolite removals than aluminium sulphate in two of three raw waters. While flow cytometry enumeration revealed a trend for increased numbers of microorganisms leading to a greater biodegradation, this was not a consistent finding. In two of three raw waters, flow cytometry live/dead analysis also identified that waters coagulated with aluminium sulphate had a higher percentage of live microorganisms. These results suggest that coagulation can influence biodegradation of taste and odours compounds and therefore further discrimination is important in order to achieve successful implementation or preservation of biofiltration capabilities within drinking water treatment plants.

Key words: taste and odour, MIB, geosmin, biodegradation, coagulation, drinking water treatment, jar testing, bioreactors, flow cytometry

Enhanced diatom growth as a method for reduction of cyanobacteria in wastewater treatment lagoons in South East Queensland

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Abstract: Management of cyanobacteria in wastewater lagoon systems is historically problematic. Diatomix is a micronutrient solution, manufactured in Australia, which uses nano-scale silica gels to transport ten micronutrients into the water and make them bio-available only to diatom algae. The level of dosing is determined by the mass of micronutrients required to correct the wastewater N: P: micronutrient ratio to that of the Redfield Ratio.

Diatomix addition has been trialled in SE QLD wastewater treatment and storage lagoons with three utility companies. The addition of Diatomix in the wastewater treatment systems has reduced cyanobacteria cell count from 75% to 99.4% over 10 weeks to 19 weeks respectively. At one site the average cyanobacteria cell count was 1,583,000 cells/mL prior to the trial starting and 197,000 cells/mL over the same period (April and May) the following year when treated with Diatomix. At a second site the cyanobacteria cell count increased from an average of 189,000 cells/mL during treatment to an average of 3,586,000 cells/mL when treatment stopped, with the first week after treatment cessation reaching 7,299,000 cells/mL. When treatment of this lagoon was re-instated ten weeks later the cyanobacteria cell count reduced to an average of 670,000 cells/mL within two weeks.

At the sites tested the average percentage of cyanobacteria cells in the total cell count was initially 85–100% (cyanobacteria cells/total cells * 100%). With treatment this percentage reduced to between 22 and 99%. In the trial where there was minimal change in the percentage of cyanobacteria within the total cell count, there was however a 78% reduction in the cyanobacterial cell count from 2,550,000 cells/mL to 566,000 cells/mL. The data does not clearly demonstrate a related increase in diatom counts at all sites and this is very likely due to two main factors. Firstly, algal cell count sampling was for suspended algae only, with no benthic samples being taken. Secondly, diatom algae are heavily grazed by zooplankton and invertebrate populations which in turn may reduce numbers counted.

Key words: cyanobacteria, diatoms, nutrients, wastewater, lagoon

The impact of draft-tube mixers on thermal stratification dynamics in Googong reservoir

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Abstract: Impeller-based draft-tube mixer systems (e.g. SolarBee and WEARS) are often used to enhance circulation in lakes and reservoirs with the intent to improve water quality. Such systems promise a much-more energy efficient alternative to the more common compressed-air bubble plume method of reservoir destratification.

Googong Dam, near Canberra, Australia, began operation of a WEARS top-down circulator system in March 2007. The system used four pumps to shift 24 $m^3 s^{-1}$ of surface layer water downwards through a 20 m-long draft tube where it was released into the reservoir. During the study period (March 2007–April 2008) the volume of the storage was approximately 60 GL with a surface area of 480 ha and a maximum depth of ca. 35 m. Thermistor chain data and water quality profiler data show the impact of the mixers on thermal stratification as well as oxygen redistribution in the water column. Following the onset of operation during the autumnal cooling period, there was a 10-day period during which the surface layer became shallower. After approximately two months the entire water column had been circulated. The system transported heat downwards throughout most of the water column during early spring. As heating became more intense at the water surface the influence of the circulators extended only to the bottom of the draft tubes. There was no significant impact of the circulators on the depth of the surface mixed layer.

Operation of the mixers raised dissolved oxygen concentrations to the depth of the draft tube but there did not appear to be an increase in the total mass of oxygen in the reservoir, i.e. the circulators appear to have redistributed the existing amount of oxygen within the water column. This implies either air-water exchange of oxygen at the water surface is a limiting factor or that the induced large increase in water temperature in the lower water column has raised in situ oxygen demand sufficiently to compensate for any increased flux into the water column across the reservoir surface.

Key words: destratification, draft-tube mixers, reservoir management, stratification, water quality

Differences in cyanobacterial strain responses to light and temperature drives species plasticity

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Abstract: Toxic *Cylindrospermopsis raciborskii* and *Microcystis aeruginosa* are two nuisance cyanobacterial species of global concern. Both species have high phenotypic plasticity, which contributes to their tolerance to environmental variables. Multiple strains exist for both species, however, the scale of the physiological variation between strains is poorly understood. In this study we examined the response of multiple strains of C. *raciborskii* and M. *aeruginosa*, to a range of light and temperature conditions.

Eight C. *raciborskii* isolates (five with straight trichomes, three with coiled trichomes) and four M. *aeruginosa* isolates, isolated from two reservoirs in Southeast Queensland, Australia, were cultured individually in a factorial design experiment with four light intensities and two temperatures. Exponential growth rate, volume of individual cells, and cell concentration at stationary phase were measured. The light attenuation coefficient (kj), as an indicator of self-shading, was calculated. A higher kj indicated higher self-shading and lower light availability.

This study showed that the intraspecific variation in exponential growth rates was greater than the interspecific variation. All the C. *raciborskii* isolates had exponential growth rates ranging from 0.15 to 0.70 day^{-1}, while M. *aeruginosa* isolates ranged from 0.16 to 0.55 day^{-1}. C. *raciborskii* isolates had a much wider variation in the surface-to-volume ratio of individual cells, and higher kj than M. *aeruginosa*. Smaller cells tended to have much lower kj within both species. M. *aeruginosa* reached higher cell concentrations under most of the treatments compared with C. *raciborskii*. However, C. *raciborskii* isolates showed greater variation in growth rates, cell volume and kj compared to M. *aeruginosa*. Interestingly, within the two C. *raciborskii* morphotypes, coiled isolates had lower kj and therefore had less self-shading, resulting in higher light availability than the straight ones.

In conclusion, this study highlights the importance of strain variability in understanding the physiology of both species, and how the co-occurrence of multiple strains can lead to high species plasticity.

Key words: growth, light attenuation coefficient (kj), cell volume, cell morphology, strain variation

GlobalHAB: A new international network putting cyanobacterial blooms on the global stage

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Abstract: The Global Harmful Algal Blooms (GlobalHAB) Programme is a cutting-edge scientific and international programme on HABs aimed at fostering and promoting co-operative research directed toward improving the prediction of harmful algal bloom (HAB) events, and providing scientific knowledge to manage and mitigate their impacts in a globally changing planet. GlobalHAB will address the scientific and societal challenges of HABs through the application of advanced technologies, training and capacity building, with a multidisciplinary approach. It will also build linkages with broader science domains (climatology, toxicology, economy, medicine, public health), emphasize social science communications and address management priorities.

Previously, global efforts in fostering cooperation on HABs focussed on marine species. However, freshwater HABs, including toxic and nuisance cyanobacteria, have now been included and provides an opportunity to more closely link and share knowledge amongst these two research and management groups. The widest international participation of the research community and stakeholders will be essential to ensure the success of GlobalHAB. Scientists working in physical, chemical and/or biological disciplines, or other fields related to harmful algal research—including the development of relevant instrumentation and models—are encouraged to contribute to this programme. Furthermore, a special invitation is made to climate, medical, economy and communication professionals and policy makers, whose expertise is fundamental to cope with the impacts of HABs in the coming years.

Key words: cyanoHABs, eutrophication, nutrients, toxic cyanobacteria

Fine-scale cryogenic sampling of planktonic microbial communities: application to toxic cyanobacterial blooms

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Abstract: A lack of fine-scale methods for sampling planktonic microbial populations hinders advancement in understanding the responses of these communities to environmental conditions. Current methods provide resolution at scales of centimetres to metres, but not at the millimetre-scale required to understand highly stratified communities. To address this, we developed two cryogenic sampling tools (cryo-samplers) to collect spatially-precise samples from aquatic environments whilst simultaneously preserving the microbial communities. The two cryo-samplers consist of metal probes which are chilled to sub-zero temperatures using liquid nitrogen. The 'Surface Snatcher' collects surface water samples which vary in thickness depending on the length of application. The 'Cold Finger' enables vertical profiles of the top 40 mm of the water column to be obtained. The frozen samples can be transported back to the laboratory on dry-ice or defrosted in the field using a specially designed cryosectioning plate. As the ice crystals which form during the cryo-sampling process disrupt the cell membrane of the specimens, conventional enumeration processes are not possible. To overcome this, we have used modern molecular techniques to enumerate cyanobacteria and functional genes using quantitative polymerase chain reaction assays and to assess cyanobacterial diversity using high-throughput sequencing (HTS).

The application of these samplers was examined in experimental mesocosms, where cyanobacterial scums (*Dolichospermum* [*Anabaena*] and/or *Microcystis*) were formed, and *in situ*, to capture cyanobacterial colonies. The Surface Snatcher was used to collect a discrete layer (ca. 1 mm) from the top of the surface water. Compared to conventional surface sampling methods, the Surface Snatcher samples contained up to 22-times more microcystin, indicating that less underlying water was incorporated into the sample. Fine-scale vertical profiles of the cyanobacterial scums were collected using the Cold Finger. These allowed us to visualise how communities of toxic and non-toxic cyanobacteria intermingle as surface 'blooms' form and extreme conditions develop in the top few millimetres – including evaluated pH (>10) and super-saturated dissolved oxygen (>150%). Using the Surface Snatcher, we also isolated individual *Microcystis* colonies *in situ*, conducted measurements under a chilled microscopy setup, determined strain diversity using HTS and assessed how these parameters relate to their cyanotoxin content.

Results from our studies on planktonic cyanobacterial communities will be presented, and we will highlight other potential applications for researchers working on dynamic microbial communities in natural environments.

Key words: *Dolichospermum*, Cold Finger, Cryo-Samplers, microcystin, *Microcystis*, Surface Snatcher

Next generation monitoring of water quality

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Abstract: Next generation sequencing (NGS) has rapidly become an invaluable molecular tool for the study of complex microbial communities. The hypervariability and multi-copy nature of the small ribosomal subunit RNA (16S rRNA) gene, coupled with the availability of growing sequence information, allow detection, relative quantification and classification of organisms (potentially) to species level.

In this context, 16S metagenomics primers with broad taxonomic coverage ("universal") have proven advantageous for the comprehensive surveys of microbiomes. Their broad specificity, however, entails that abundant taxa will represent a large proportion of the sequence data, while rarer taxa (possibly including pathogens, bioindicators or other target groups of interest) may remain undetected, depending on the sequencing depth implemented. In the present study, two novel 16S rRNA primer pairs against the phylum cyanobacteria were designed for NGS-based applications, in the context of water quality studies. The novel primers were extensively tested and compared to widely-adopted universal 16S rRNA primers, starting from cyanobacterial cultures and heterogeneous fresh-water samples.

Compared to the universal 16S metagenomics primers, both *in silico*– and wet– analyses demonstrated that the novel primers showed increased specificity for cyanobacteria and proteobacteria, allowing detection, identification and relative quantification also of toxic bloom-forming microalgae, water-quality bioindicators and pathogens.

Cyanobacteria and proteobacteria formed a proportion of the sequences amplified by the novel primers about two-fold larger than that obtained with the universal 16S rRNA primer set. In molecular surveys with a specific focus on problematic groups like cyanobacteria and proteobacteria, the increased selectivity of the novel primers for these taxa allows parallel sequencing of more samples per run, compared to the universal primers. This brings clear economic benefits but still guarantees coverage and species discrimination for the target groups.

Key words: next generation sequencing, pathogens, cyanobacteria, bioindicators, water quality, 16S, microbiome, proteobacteria, monitoring, DNA

The development of the PhytoxigeneTM CyanoDTec test from research to implementation as a cyanobacteria screening tool for prediction, monitoring and management of harmful algal blooms in public water supplies

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Abstract: It is now well over a decade since the genetic basis for biotoxin production in cyanobacteria was identified and characterised. The discovery of these toxin biosynthetic pathways was expected to enable the rapid development and subsequent implementation of genetic screening methods for identifying toxin-producing cyanobacteria in public water systems. The uptake of this application, however, has been limited, but along with the development of a standardised commercial assay and a number of significant harmful algal events, utilisation and integration into water testing programs has now begun.

In August 2014 Toledo City issued a “Do Not Drink” advisory to its citizens. For over 5 days half a million people were told not to use any tap water. The microcystin that caused this advisory was produced by a large cyanobacterial bloom on Lake Erie. In 2015 Lake Erie once again bloomed in what is now believed to have been the largest bloom ever recorded. This was followed by a 1,000 km bloom over the full length of the Ohio River. These events caused significant debate and resulted in an overhaul of the water testing guidelines by the Ohio EPA. During this process the agency evaluated and then proposed a molecular test as an alternative to their traditional taxonomic screening and cell counting.

On June 1, 2016 the new rules for water testing became law, and for the first time a state authority has adopted a genetic approach to screening for toxin-producing cyanobacteria. The Ohio EPA has chosen to use the Phytoxigene CyanoDTec assay, a multiplex quantitative PCR that simultaneously detects, discriminates and quantitates the genes for total cyanobacteria, and the production of microcystin and nodularin (*mcy/nda*), cylindrospermopsin (*cyr*) and saxitoxin (*sxt*). The presentation will look at the background and process in which this decision was made and how it is being utilised within their guidelines.

Key words: cyanobacteria, biotoxin pathway, cyanodtec, Phytoxigene, microcystin, saxitoxin, screening

ORAL ABSTRACTS – Day 2

Keynote Address 2 – Rachael Poon

Applying cyanobacteria risk management – A Victorian perspective

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Abstract: Research has increasingly shown the highly variable nature of cyanobacterial blooms and a myriad of factors which influence cyanobacterial cell proliferation and toxin production. Regulators rely on National guidance documents such as the *Australian Drinking Water Guidelines* and the *Guidelines for Managing Risks in Recreational Water* to provide a framework for managing these risks in drinking water and recreational water bodies. Under Victorian legislation, drinking water suppliers are required to assess cyanobacterial risk to drinking water through the use of risk management plans. Local water managers are assigned responsibility for the management of cyanobacteria in publicly accessible recreational water bodies through the Victorian Blue-Green Algae coordination framework. Blooms are assessed on a case by case basis using the best available science. When dealing with issues relating to exposure to cyanobacteria and cyanobacterial toxins, the lack of epidemiological data and research on cyanobacterial dose response means there is a limited understanding on potential adverse health outcomes. Regulators and water managers continually evolve their implementation of the preventive risk management framework in light of the best available scientific evidence. Recent Victorian case studies illustrate some of the issues faced by regulators and water managers in responding to cyanobacterial blooms.

Key words: Victoria, Cyanobacteria, blue-green algae, public health, drinking water, recreational water, regulation

Managing cyanobacterial toxin risks to recreators: a case study for Cylindrospermopsis raciborskii dominated inland lakes in south east Queensland

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Abstract: Seqwater is responsible for South East Queensland's bulk water supply and long term planning of the region's future water needs. Included in this portfolio is the management of recreational activities on Seqwater's lakes. Seqwater currently provides on-water recreational access to 17 lakes for activities such as: boating (motorised and non-motorised), fishing, water-skiing and swimming. These 17 lakes are the main sites in South East Queensland (SEQ) for recreation on/in freshwater, and provide a recreational landscape of regional significance.

The use of drinking water lakes for recreational activities can adversely affect water quality, not only for drinking water supply, but also ecological function and the on-going suitability for recreation. Those potential adverse effects must be weighed against the benefits to health and wellbeing of recreational water use (e.g. rest, relaxation and exercise) and the positive impacts on local economies that rely on water-associated recreational activities. Seqwater understands this important balance, which is reflected in Seqwater Strategic Vision: Healthy communities, Prosperous region.

In order for Seqwater to continue to deliver on their vision and minimise risk to recreators, a Recreational Water Quality Management Plan has been in operation for the last five years to balance access to recreation opportunities while protecting natural resources and water quality. Seqwater's recreational lakes are situated in open catchments with associated microbial risks, nutrient and sediment mobilisation risks. Many catchments also have standing populations of potentially toxic cyanobacteria (naturally occurring), that can bloom over summer and alter water quality in the lakes. There are minimal guidelines and advisory documents for water authorities to guide decision making processes for recreational waters in Australia, particularly compared with the treated water industry. Those that do exist focus on southern systems, dominated by saxitoxin-producing species, and are not directly transferrable. . In South East Queensland, the most prevalent potential toxin producing species are: *Cylindrospermopsis raciborskii*, *Dolichospermum circinale* and *Microcystis aeruginosa*. The Cylindrospermopsin-producing C. *raciborskii* tends to dominate bloom events with nearly all Seqwater storages having a Medium/High annual cyanobacterial susceptibility score.

Seqwater has developed a range of cyanobacterial biovolume, cell count and toxin level triggers to drive the specialised monitoring program targeting multiple potentially toxic species. The authority undertakes extensive water quality monitoring at recreational water storages to ensure the water is fit for the approved recreational activity. Following a four part cyanobacterial escalation/de-escalation system, Seqwater is able to track recreational water quality suitability and close lakes to recreators when waters become unsafe. Because Seqwater permits recreation despite the known inherent water quality risks, the closure of lakes should not be the point of focus in communication with the recreating general public. It should instead be communicated that Seqwater is opening lakes to recreation when conditions are suitable and the recreators' safety will not be compromised, in line with Seqwater's legal obligations.

Key words: recreation, management, monitoring, cyanobacterial triggers, open catchments, source water quality

Should we be controlling nitrogen or phosphorus to manage cyanobacterial blooms? Lessons from studies of *Cylindrospermopsis raciborskii*

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Abstract: It is well established that increased nutrient loads are linked with a higher probability of cyanobacterial blooms. However, debate still rages in the scientific literature on whether catchment sources of nitrogen, phosphorus or both should be reduced to manage blooms. We have studied the relationship between nutrients and the toxic cyanobacterium, *Cylindrospermopsis raciborskii* in laboratory, mesocosm and field studies to build a picture of the complex strategies employed by this organism, and examine the implications for management and prediction. It is clear from our laboratory studies that C. *raciborskii* is highly adapted to low and variable phosphorus concentrations, using a range of physiological strategies. Low phosphorus availability typically occurs during periods of summer stratification. However, phosphorus addition does not increase growth rates, although there is evidence the population shifts towards a higher proportion of toxic species. It has been argued that controlling nitrogen input loads to waterways is irrelevant for nitrogen fixing cyanobacteria such as C. *raciborskii*. However, it is clear from our studies that dissolved forms of nitrogen can increase growth rates compared with nitrogen fixation, meaning that cells can reach a higher biomass sooner. Additionally, C. *raciborskii* can fix nitrogen, they cannot rapidly switch between the use of dissolved forms of nitrogen and nitrogen fixation. There is also evidence that production of the heterocysts required to fix nitrogen can be limited by the availability of phosphorus. Therefore, strategies deployed by this organism for use of nitrogen and phosphorus differ, and it is also important to consider the interaction between the two nutrients. In terms of the implications of this work, it points to the need to manage phosphorus in order to control toxin levels, and nitrogen to control growth.

Key words: nitrogen, phosphorus, cyanobacterium, nitrogen fixation, toxins, cylindrospermopsin

Cyanobacterial problems in an iconic lake – Lake Burley Griffin, Canberra

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Abstract: Lake Burley Griffin was designed as the centrepiece of the new National Capital by the American architect who won the design competition for the city. It was 40 years before excavation commenced. The lake is retained by a dam of height 33 m, holding 33,000,000 m^3 of water (33 GL). The inflow is largely from the Molonglo River, which drains an old mining area and receives the outfall from the Queanbeyan Sewage Treatment Plant. There are also several urban creeks, draining highly developed residential as well as commercial areas. The unfortunate consequence was that the total phosphorus concentrations in much of the Lake reached over 0.1 mg/L at intervals since filling in 1964, with almost annual water blooms of cyanobacteria.

The predominant organisms in mid-late summer were (and still are) *Microcystis aeruginosa* at intermittent concentrations over 100,000 cells/mL, with scum formation. In the cooler months extensive water blooms of *Dolichospermum (Anabaena) circinale* occur, with occasional dog mortality after swimming in a scum.

In the mid-1980s the upstream Sewage Treatment Plant was modified to reduce phosphate discharge, with the result that the phosphorus concentrations dropped over a decade to range from 0.02–0.06 mg/L. This initially appeared to reduce cyanobacterial blooms, but between 2007 and 2010 there was a resurgence of blooms with concentrations again reaching close to 100,000 cells/mL Between 2006 and 2008 Canberra received very low rainfall, which returned to flooding in 2010.

At the phosphorus concentration of around 0.06 mg/L in the East Basin there was sufficient phosphorus to support a water bloom under clear water and mild weather conditions. An anoxic reach just above this shallow basin may have exacerbated the situation, as well as a high cyanobacterial concentration between the Sewage Treatment Plant and the Lake.

The Safety Guidelines in place for Lake use close body-contact water sports at cyanobacterial concentrations above 50,000 cells/mL, and the Lake has been closed in summer repeatedly when recreational use is maximal and competitive body-contact water sports are held. Even an international triathlon was cancelled.

Several efforts are underway to improve the situation, including replacement of the Sewage Treatment Plant, constructing sedimentation ponds, swales and wetlands on the urban creeks and re-use of stormwater. These will be discussed in the light of overseas experience.

Key words: cyanobacterial water blooms, phosphate concentration, health and safety, amelioration methods

The interactive effects of flow and nitrate supply on *Phormidium* accrual

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Abstract: Toxic benthic cyanobacterial proliferations, particularly of the genus *Phormidium*, are an escalating problem worldwide in freshwater environments and associated animal toxicosis events are being reported with increasing frequency. *Phormidium* proliferations are particularly problematic in New Zealand rivers, where there has been a marked increase in the distribution, intensity and frequency of proliferations in recent decades. Ingestion of *Phormidium* mats has resulted in over100 dog deaths in New Zealand in the last decade. Previous observational studies have indicated a range of environmental factors as potentially important in determining patterns of *Phormidium* occurrence, but manipulative experimental approaches are required to disentangle and identify the causal mechanisms underlying these patterns. *Phormidium* mats are common in streams draining agricultural catchments, and elucidating the individual and synergistic effects of nitrate concentration and flow velocity on bloom development, are an important consideration for both fluvial science and management. We conducted a streamside channel experiment that evaluated the responses of *Phormidium*-dominated mats in terms of biomass (chlorophyll a and phycoerythrin), patch expansion, cellular nutrient concentrations and taxonomic composition to three different nitrate levels, crossed with two flow levels, resulting in a total of six treatments each replicated three times. The 20 day experimental period encompassed the *Phormidium* mat accrual cycle from initial colonisation, growth and detachment by sloughing. Preliminary statistical analysis highlighted significant differences between *Phormidium* biomass accrual between treatments. Growth enhancement was most pronounced on day 4 and day 16, where the *Phormidium* mats in treatments with high flow and highest nitrate concentration had a higher biomass than all other treatments. The study highlights the complex and interactive relationships between flow and nitrate and benthic *Phormidium* accrual cycles.

Key words: *Phormidium*, streamside experimental channels, flow, nutrients, patch expansion

Enumerating toxic *Phormidium autumnale* in environmental samples

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Abstract: Benthic cyanobacterial blooms can pose a significant risk to the ecological health of freshwater ecosystems. *Phormidium* spp. can produce powerful neuromuscular blocking compounds collectively known as anatoxins. Like other cyanobacteria, *Phormidium* spp. comprise both toxic and non-toxic genotypes, which are morphologically identical. Toxin concentrations from *Phormidium* spp. are usually calculated based on the total weight (wet or dry) of samples. As mats contain both toxic and non-toxic genotypes, and other organic and inorganic material it is impossible to identify whether increases in anatoxins are the result of upregulation in toxin production, or a greater proportion of toxigenic cells in a sample. The *ana*C gene from the anatoxin biosynthesis pathway is single copy in *Phormidium* spp. and is completely absent in non-toxic genotypes. We developed primers and a Taqman probe from *ana*C gene sequences in *Phormidium autumnale*. Quantitative PCRs were run on environmental samples and compared to a standard curve to determine the number of toxigenic cells in the sample. Toxin quotas per toxigenic cell were calculated and compared to a suite of environmental data. This method enables an accurate determination of anatoxin quota in *P. autumnale* by relating toxin production to toxigenic cells. Knowledge on how environmental factors influence toxin quota and genotype ratios will assist in on-going management and risk assessment of toxic benthic cyanobacterial blooms.

Key words: quantitative PCR, *Phormidium autumnale*, toxin quota, anatoxin

Acute oral toxicity of deoxy-cylindrospermopsin in male Balb/c mice

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Abstract: Deoxy-cylindrospermopsin (D-CYN) is a chemical analogue and biosynthetic precursor of cylindrospermopsin (CYN). An initial *in vivo* toxicity study reported in 1999 concluded that D-CYN was not toxic to mice, but subsequent *in vitro* investigations have revealed comparable biological activities to CYN. Employing a humane endpoints acute toxicity model of regulated hypothermia and sickness behaviour, we dosed male Balb/c mice ($n = 6$) with D-CYN by oral gavage at 6 mg/kg body weight for five consecutive days, with an investigator-blind treatment for the initial dosing and monitoring periods. Negative control mice ($n = 4$) were dosed with HPLC-grade water that was used to reconstitute mobile phase eluate collected under identical chromatographic parameters as employed for our isolation and purification of D-CYN. Positive control mice ($n = 6$) were exposed to pure CYN given as a single gavage dose, also at 6 mg/kg.

Positive control mice all showed signs of acute CYN intoxication, commencing some 40 hours after dosing, eventually manifesting profound hypothermia (to 27°C). At necropsy, livers were pale and enlarged. Both negative control mice and D-CYN-dosed mice showed no signs of sickness behaviour and were normothermic throughout the study. No gross abnormalities were seen in either group at necropsy.

Relative liver weights across the three groups differed at the 5% significance level: 1-way ANOVA F=38.94, $p < 0.0001$, with post-hoc tests showing that mean liver weights of CYN-dosed mice were significantly larger than livers of both D-CYN and water-dosed mice, confirming our observations at necropsy of hepatomegaly in the CYN treatment group. No significant differences in relative kidney weights were seen across the three treatment groups (1-way ANOVA F=0.923, p=0.42). Repeated-measures ANOVA of rectal temperatures also revealed statistically-significant differences: F=8.69, p=0.001, with post-hoc tests confirming that mean CYN group temperatures were different to both water and D-CYN group temperatures. Rectal temperatures in the D-CYN group mice were not significantly different to those of the negative controls.

D-CYN from D-CYN-dosed mice and CYN from CYN-dosed mice was identified and quantified by HPLC-MS/MS in urine, faeces and liver, but neither compound was seen in blood or brain tissues. Analysis of kidney tissues is pending.

Earlier proton NMR work on the batch purification of D-CYN used for this study has shown it to be a pure compound, with the uracil moiety in the keto configuration. We conclude that D-CYN in this keto form is non-toxic to mice by the oral route at five daily doses of 6 mg/kg, compared to a single equivalent dose of CYN which is highly toxic, causing acute liver failure. We aim to continue investigating this apparent disparity between the *in vivo* toxicity and *in vitro* activities of D-CYN.

Key words: cyanobacteria, cyanotoxin, toxicology, cylindrospermopsin, deoxy-cylindrospermopsin, gavage, humane endpoints toxicity testing

Intraspecific variation in growth, morphology and toxin quotas for the cyanobacterium, *Cylindrospermopsis raciborskii*

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Abstract: *Cylindrospermopsis raciborskii* is a bloom forming cyanobacterium with complex population dynamics and toxicity. The range of geographical distribution or dominance of C. *raciborskii* appears to be expanding and it also reflects on its associated toxin productions and yield. C. *raciborskii* strains isolated from several geographical locations within a region or country show considerable variation in growth and toxin production response to environmental conditions. Such variations reflect a highly adaptive strategy and are speculated to be the main driver for the rapid geographic expansion of C. *raciborskii*. However, the scale at which this diversity exists is unclear, including within a single population in a waterbody.

In January of 2013 a single sample was collected from surface waters in Lake Wivenhoe, Australia, and twenty-four individual trichomes were isolated. There was diversity amongst the isolates in terms of growth rate, toxin quota/ratios and morphology despite a lack of phylogenetic differentiation as determined by HIP1 PCR. These results suggest a population with highly adaptive isolates, rather than a population containing multiple strains with possible introductions from different locations.

The substantial physiology variation was seen in a 2.5-fold difference in cylindrospermopsin cell quota (QCYNs). Relative to cell biovolume, CYNs concentrations exhibited a 10-fold difference between isolates, with CYNs varying from 0.76 to 7.53 fg μm^{-3}. Euclidean distance analysis grouped isolates by CYN and deoxy-CYN (CYN:dCYN), QCYNs, growth rate or size. The intraspecific variation in QCYNs, in combination with differences in CYN:dCYN ratios, would have a large effect on bloom toxicity and toxin yield, depending on the strains present. Therefore, differences in the QCYN among isolates reflects that population dynamics are responsible for the measured variation in toxin yields of blooms.

The results from this study suggest that diversity in response to environmental conditions exists even on the microscale. It is unclear which environmental conditions would favour one isolate over another, and this is an area warranting further work. This micro-scale diversity has further implications for predicting and mitigating harmful cyanobacteria blooms and their ecological interactions.

Key words: cyanobacteria, cylindrospermopsin, ecotype, harmful algae blooms, morphology, strain

Risky rivers: identifying river susceptibility and factors that promote benthic *Phormidium* proliferations

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Abstract: Proliferations of the benthic anatoxin-producing cyanobacterium *Phormidium* are increasing in prevalence in cobble-bed rivers worldwide. This is of particular concern when these rivers are used as drinking water sources or for recreation. Knowledge on the variables promoting proliferations is limited and based on data from a few rivers. In this presentation we describe our recent work aimed at identifying what makes a river susceptible to *Phormidium* proliferations and factors that promote proliferations. *Phormidium* coverage data was obtained from over 500 sites distributed nationally and data on river features, catchment and land use for each site sourced from national databases. A combination of boosted regression tree and generalised additive models (GAMs) were used to develop a statistical model to predict the susceptibility of rivers across New Zealand to *Phormidium* proliferations. To obtain a more in-depth knowledge of drivers of bloom formation we assessed *Phormidium* cover, physicochemical variables and anatoxin concentrations at ten sites in seven New Zealand rivers every week for two years. GAMs identified dissolved inorganic nitrogen (DIN) over the accrual period up to 0.8 mg L^{-1}, accrual dissolved reactive phosphorus below 0.005 mg L^{-1}, water temperatures greater than 15°C, and conductivity as having a positive and statistically significant effect on *Phormidium* cover. Flow intensity, expressed relative to the long-term median, also had a positive effect up to 0.4 times the median flow and a negative effect when greater than 0.5 times the median. GAMs identified strong relationships between elevated toxin concentrations and low conductivity and increasing *Phormidium* cover, and significantly lower toxin concentrations when DIN was below 0.2 mg L^{-1}. These data demonstrate that multiple physicochemical variables influence *Phormidium* proliferations and toxin concentrations, and indicate that the relative importance of these differs among rivers and sites. We will briefly discuss on-going research which aims to use manipulative experiments to confirm observations from correlative studies, and to improve knowledge on *Phormidium* and anatoxin toxicity.

Key words: anatoxins, *Phormidium,* proliferations, rivers

A novel nodularin producing cyanobacterium from Edgbaston Reserve, north-eastern Australia

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Abstract: A novel nodularin producing benthic cyanobacterium *Iningainema pulvinus* gen nov., sp nov. was isolated from a freshwater ambient spring wetland in tropical, north-eastern Australia and characterised using combined morphological and phylogenetic attributes. It formed conspicuous irregularly spherical to discoid, blue-green to olive-green cyanobacterial colonies across the substratum of shallow pools. Morphologically *Iningainema* is most similar to *Scytonematopsis* Kiseleva and *Scytonema* Agardh ex Bornet & Flahault. All three genera have isopolar filaments enveloped by a firm, often layered and coloured sheath; false branching is typically geminate, less commonly singly. Phylogenetic analyses using partial 16S rRNA sequences of three clones of *Iningainema pulvinus* strain ES0614 showed that it formed a well-supported monophyletic clade. All three clones were 99.7–99.9% similar, however they shared less than 93.9% nucleotide similarity with other cyanobacterial sequences including putatively related taxa within the Scytonemataceae. Strain ES0614 formed a novel branch in the *nifH* phylogeny which was not associated with any major clade. Amplification of a fragment of the *ndaF* gene involved in nodularin biosynthesis from *Iningainema pulvinus* confirmed that it has this genetic determinant. Consistent with these results, analysis of extracts from strain ES0614 by HPLC-MS/MS confirmed the presence of nodularin at concentrations of 800–1096 µg g^{-1} dry weight. This is the third genus of cyanobacteria shown to produce the cyanotoxin nodularin and the first report of nodularin synthesis from the cyanobacterial family Scytonemataceae. These new findings may have implications for the aquatic biota at Edgbaston Reserve, a spring complex which has been identified as a priority conservation area in the central Australian arid and semiarid zones, based on patterns of endemicity.

Key words: phylogeny, taxonomy, cyanotoxin, benthic, tropical, 16S rRNA, *nifH*, *ndaF*, ambient springs

POSTER ABSTRACTS

Development of a satellite based algal bloom alert early warning system for New South Wales

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Abstract: The occurrence of cyanobacterial blooms is increasing across the globe and results in significant economic impact to affected communities. Toxic cyanobacterial blooms are a particular concern, increasing threats to both human and animal health. Traditional field monitoring for algal bloom detection involves identification and cell counting which, whilst reliable, imparts a lag time and can be limited in spatial extent. In the Australian context, as with other developing *and* developed countries, overcoming the vast geographic distances to be covered is a particular challenge; satellites and other forms of remote sensing may provide an appealing complement to water managers for water quality monitoring. Recently launched satellite sensors offer the potential of high resolution, wide scale and frequent monitoring of water quality in inland water bodies of a range of sizes in support of the development of early bloom alerts for water managers.

We report on continued Australian strategic investment in inland water quality remote sensing to develop tools to assess and visualise algal blooms across larger spatial scales to support cyanobacterial monitoring. The tool is built upon the Australian Geoscience Data Cube (AGDC) for DPI Water. The AGDC represents a 19 year time series of consistently pre-processed Landsat data for the Australian continent. As new image acquisitions are loaded up to the AGDC in near real time they are processed with a standardised turbidity algorithm in the first instance. The results can then be visualised for NSW at state level to provide a rapid state-wide overview of overall algal alert status, or at the scale of the individual water body to allow the determination of spatial bloom dynamics. Current data can be displayed with historical data to allow the up to date situation to be put into a longer term context.

The presentation will outline the AGDC and the processing and visualisation systems that have been produced, and its power in helping management decision making on algal bloom risk across wider spatial areas. The future potential of such a system will also be highlighted as new sensors systems (e.g. Sentinel 2) come on line allowing the ability to exploit improved algorithms.

Key words: earth observation, cyanobacteria, algal blooms, water quality, remote sensing, visualisation, information services, early warning, satellite sensors

Application of first order rate kinetics to explain changes in bloom toxicity–the importance of understanding cell toxin quotas

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Abstract: How environmental factors trigger changes in bloom toxicity is a perennial question that has been studied for about 150 years. In general, the results have been disparate–largely due to misinterpretation of results caused by the casual way that terms like toxin production, toxin concentration, toxicity and yield have been used interchangeably. Understanding how cell toxin quotas (Q_{toxin}) are controlled at the cellular level provides the key to understanding what drives changes in bloom toxicity and toxin yield at the environmental level.

First order rate kinetics can be used to compare rates of processes. Orr and Jones[1] (1998) used first order rate kinetics to show that a strong linear correlation exists between the specific rate of cell division (µc) and the specific rate of microcystins production (μ_{MCYST}) by *Microcystis aeruginosa* under nitrogen-limited growth. The consequence of this finding is that the amount of MCYSTs present in an individual cell (i.e. the cell MCYSTs quota - Q_{MCYST}) is essentially fixed or will only vary within a small (2–3-fold) range for each strain. This range can be explained by redistribution of the existing MCYSTs pool into a larger number of cells during cell division. Analysis of previous research showed this relationship also holds true for production of MCYSTs by both *Oscillatoria agardhii* and *Anabaena* spp. irrespective of what the environmental variable was. However, we know that different strains can have QMCYST that differ by more than two orders of magnitude from as low as 0 up to more than 200 fg cell^{-1}.

We have recently shown a similarly strong linear correlation between the growth rate (*sensu strictu*) and the rate of production of cylindrospermopsins (CYNs) by *Cylindrospermopsis raciborskii* for a suite of environmental drivers, including nutrients, temperature and light. It appears that for some of these species at least, production of their toxins is constitutive (produced in relatively constant amounts in all cells without regard to cell environmental conditions) and is not triggered.

For both *M. aeruginosa* and *C. raciborskii* changes in bloom toxicity is controlled by environmental variables through the effect those environmental variables have on the population dynamics and strain dominance of strains with different Q_{toxin}, and not though any trigger mechanism that switches toxin production on and off at either the molecular or the cellular level.

Acknowledgments: Seqwater, Australian Research Council, Technical Staff

References: PT Orr and GJ Jones (1998) Relationship between microcystin production and cell division rates in nitrogen-limited *Microcystis aeruginosa* cultures. Limnol Oceanogr 43(7) 1604–1614.

Keywords: microcystins, cylindrospermopsins, bloom, toxicity, toxin production

Wastewater microbiome assemblages and the effect of abiotic factors on cyanobacterial blooms

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Abstract: The metabolic activity of microorganisms is essential for the removal of nutrients, pollutants and pathogens from municipal wastewater. However, the efficiency of this removal depends on the complex and mutable interplay between biotic and abiotic variables occurring in the treatment plant.

During the present study, samples were collected from three waste stabilisation ponds and an oxidation ditch system in Western Australia. Three abiotic variables were selected for testing–season, treatment stage and treatment technology–after hypothesizing that the microbial assemblages of Bacteria, Archaea and Eukarya would respond to them accordingly. A spatial-temporal analysis of the microbiome was conducted by next-generation DNA sequencing and the results were compared to the efficiency of removal of various nutrients and micropollutants and the presence of problematic cyanobacteria species.

Multivariate analysis allowed the clustering of microbiome and chemical fingerprints, based on season, treatment stage and treatment technology. Influents showed consistent profiles, irrespective of the abiotic variables. Conversely microbiome and chemical fingerprints in later stages and across seasons and technologies were more variable, with treatment stage and plant design having a bigger effect on the community than season. Identifying the most important variables correlated with particular biological assemblages may allow prediction of, not only the fate of pathogens, bioindicators, cyanobacteria and beneficial microbial groups, but also their activity and, ultimately, the plant performance.

Key words: next generation sequencing, pathogens, cyanobacteria, micropollutants, water quality, 16S, microbiome, proteobacteria, monitoring, DNA, wastewater

Cyanobacteria cell lysis during oxidation: development and application of a cell integrity analytical method

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Abstract: Harmful cyanobacterial blooms in drinking water sources are a growing challenge for drinking water production. Although conventional treatment processes are highly effective for removal of intact cells, the presence of high cell concentrations typical of bloom conditions at water intake of treatment plants can overwhelm treatment capacities and lead to breakthrough of cells into post-oxidation process. Pre-oxidation of cells in raw water and inter-oxidation of settled water can oxidize cyanobacterial cells and their associated toxins and taste and odour compounds. However, scarce and conflicting evidence on the impacts of oxidation on cyanobacterial cells have been reported, namely regarding: the level of lysis or changes in cell properties. Furthermore, a systematic study to assess and select the best cell integrity assessment method is not available. The objectives of this project were to (1) develop the methodology for flow cytometry analysis using different fluorochromes for cyanobacteria cell viability assessment, (2) select the most precise and practical method, and (3) use the selected method to investigate the cell (at different stages of growth) lysis during oxidation.

Three flow cytometry methods were developed: (1) “FDA and PI”: FDA (fluorescein diacetate) for live cells and PI (propidium iodide) for compromised cells; (2) “BacLight®”: SYTO9 for live cells and PI (propidium iodide) for compromised cells; and (3) “SYTOX Green®”: Compromised cells. Laboratory cultures of *Dolichospermum circinale* (formally known as *Anabaena circinalis*), *Cylindrospermopsis raciborskii* and *Microcystis aeruginosa* were used for cell integrity method development and comparison. Ozonation and chlorination was used for investigating the cell viability after oxidation in bench-scale experiments. For each experiment, 100 000 cells/mL of cyanobacterial cells were subjected to three oxidant doses. Samples were taken at predetermined contact times to measure cell viability (using the selected method), conduct cell count and dissolved organic carbon analysis. Apparent rate constants of loss of cell viability were then calculated.

This presentation will discuss the advantages and limitations of each flow cytometry method. “FDA/PI” was selected as the most precise and practical method. Shape of cells and their filaments play key roles in selection of the method. This method provided the opportunity to calculate the CT values required for cell lysis during oxidation. A wide range of CT values (18–76 mg·min/L) were required to result in complete loss of cell viability depending on the species. Interestingly the loss of cell viability did not always correspond with significant decrease in total cell numbers. Notably, 15% to 37% of cells were viable after pre-ozonation considering the fact that ozone is a strong oxidant agent. Cell resistant to oxidation varied based on species and growth phase. These data demonstrated the knowledge gaps in available information regarding the cell integrity during oxidation. These data help the researchers and utilities to understand the scope of cyanobacterial oxidation challenges and to identify the future research direction.

Key words: cyanobacteria, flow cytometry, cell lysis, cell integrity, oxidation, chlorination, ozonation

Smart cyanobacterial monitoring techniques for real-time management of bloom events and treatment adjustment

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Abstract: Cyanobacterial blooms and their harmful metabolites i.e. toxins and taste and odour (T&O) compounds, are being detected more frequently in drinking water sources, leading to breakthrough and accumulation of cells, toxins and T&O compounds within drinking water treatment plants. Additionally, detection of unacceptably high concentrations of cyanotoxins and/or T&O compounds at the water intake and/or treated water has led to instances of advisories against drinking the tap water across the world, including Australia. Currently, the water industry relies on cyanobacteria analysis with 24 to 72 hours of delay which lessens the adequate respond time. Online fluorescence probes are available for the rapid detection of cyanobacterial presence and on time treatment adjustment to ensure efficient removal of cells and toxins. However, researchers and utilities are facing several questions regarding the function of these probes, selection of a suitable probe, difficulties and biases involved with their application and their benefit for management purposes.

This project aims to:

- Demonstrate the function of fluorescence probes, provide detail information about the available probes and their advantages and limitations including the bias involved with their measurement, and present a guideline to select a probe suitable for site-specific needs
- Assess the applicability of these probes for management purposes and trigger action, and present novel installation within full-scale water treatment plants for operational application of these probes.

Laboratory and field validation of assays showed that fluorescence probe readings were sufficiently accurate. However, sources of interferences were identified with probe measurements which included (a) the presence of chlorophyll-a from other phytoplankton and mixed phytoplanktonic population, (b) water turbidity and light scattering particles, (c) varying cyanobacterial cell size, biovolume and cellular agglomeration, and (d) optic probe calibration. Correction procedures were developed for the identified sources of interferences and validated during field monitoring. Fluorometric probes were used for decision making during water treatment plant operation and have been applied as a management technique for treatment adjustment for the first time in Australia. This project is part of the ongoing ARC Linkage Project research strategy at UNSW Australia and concluded that probe users should be fully aware of the sources of interferences when interpreting the in situ probe measurements.

Key words: cyanobacteria, water treatment, real-time management, fluorescence probe, sources of interferences

Bioelectrochemical detection of Cyanobacteria as a tool for harmful bloom management

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Abstract: Cyanobacteria are amongst the oldest microorganisms on earth, possessing valuable competitive advantage over other aquatic phytoplanktonic species. Eutrophication and increasing temperatures have been favouring the periodic proliferation of harmful cyanobacterial blooms both in planktonic and benthic environments, resulting in increased turbidity and limitations to light penetration in the water column that have catastrophic effects for aquatic ecosystems. Furthermore, the production of toxins that occur naturally as secondary metabolites are also a major concern for public health. In order for effective policies and management strategies to be set in motion, it is imperative to effectively monitor cyanobacterial populations in situ (1). In this work, a novel detection and monitoring method based on photocurrent measurement is proposed.

Various cyanobacteria species have demonstrated the ability to undertake extracellular electron transfer (EET) to insoluble acceptors – e.g. to reduce oxidative stress caused by excessive light exposure – and are thus able to produce a measurable electrical current (2).

Using a three-electrode photoelectrochemical cell, with a working electrode (graphite rod) poised at a potential of +0.6V vs. SHE under light/dark cycles, we are able to detect photocurrent produced by harmful cyanobacteria Anabaena circinalis. The electrode surface was modified by electro-polymerisation of a redox mediator (methylene blue), which has been shown to enhance anodic current output of photosynthetic consortia. Current output was confirmed through chronoamperometry immediately after inoculation and a clear profile was obtained in synchrony with the light/dark cycles.

These preliminary results indicate that it may be feasible to use a bioelectrochemical photocurrent-based system to monitor cyanobacteria. The establishment of a correlation between current generation and cyanobacterial biomass density, and even the potential profiling of an electroactive population of microorganisms based on divergent responses of species to specific electrode potentials and materials constitutes an exceptional opportunity for developing a powerful tool for the detection/monitoring of cyanobacteria in e.g. water reservoirs.

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Key words: cyanobacteria monitoring, bioelectrochemical sensing, amperometry.

Cyanobacteria in the inundated wetland soil

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Abstract: Microbial communities provide vital metabolic processes in inland floodplain wetlands. Due to their micron-scale size, soil biodiversity distribution and dynamics could be better understood (their structural complexity can only be achieved) using genetic methods. We studied the diversity and composition of the bacterial community in sediments collected from 50 locations at two sites in the Macquarie Marshes using Illumina sequencing of the bacterial 16S rRNA gene. Soil samples collected from marsh sites with varying flood frequency were inundated in the laboratory. Of the microbial community identified from the inundated samples, Cyanobacteria formed a minor component, relative to other bacterial phyla like Proteobacteria and Actinobacteria. The spatial distribution of Cyanobacteria did not show a simple relationship with the past flood frequency. Further analysis of cyanobacterial spatial dispersion with soil carbon, phosphorus, nitrogen and other elements should be considered to explain their spatial occurrence and to better understand the hydro-geomorphic and biogeochemical processes in the Marshes.

Key words: Cyanobacteria, inland floodplain wetlands, inundation frequency

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